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14. ABSTRACT

In the first year of this project we have cloned microRNAs from two prostate cancer cell lines, PC3 and LnCAP, the latter from cells grown in the absence and presence of androgens. The clones have been subjected to sequencing and the sequences are being deconvoluted to identify the cloned microRNAs. We have also prepared microRNAs from LnCAP cells plus and minus androgens and established conditions to hybridize them to microarrays of microRNAs in an attempt to determine which microRNAs are up- or down-regulated by androgen treatment.

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MicroRNAs, prostate cancer, microarray, gene expression, androgen

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INTRODUCTION:

Our objective is to identify the microRNAs that are expressed in three different prostate cancer cell lines, PC3, DU145 and LnCAP. We also would like to characterize microRNAs that are induced or repressed when an androgen responsive prostate cancer cell line, LnCAP is grown in the presence and absence of androgen. Comparison of the microRNA expression patterns will identify microRNAs that are specific to certain cancer cell lines and that may have a role in the cell's response to androgens.

BODY:

In the Statement of Work we had projected that tasks 1.1 to 1.3 will be completed by 12 months

Task 1.1: Grow DU145, HeLa and HCT116 cancer cell lines; isolate size-selected microRNAs from each of them. Grow LnCAP cells in the absence and presence of androgens and isolate size-selected microRNAs

Status: We have prepared microRNAs from LnCAP cells in the absence and presence of microRNAs. Our major emphasis has been on troubleshooting for task 1.2 and so we have not yet purified microRNAs from DU145, HeLa or HCT116 cells.

Task 1.2: Ligate adapters, make cDNAs of the microRNAs, clone the cDNAs to make libraries representing microRNA complements, and sequence the clones in the libraries.

Status: We now have libraries of microRNAs from LnCAP cells before and after androgen treatment. For the better part of the year, we had great difficulty in obtaining any clones of microRNA concatamers. There were problems at multiple steps of the protocol that had to be identified and resolved:

(a) Yield of microRNAs isolated after size-fractionation was very low. We labeled a synthetic microRNA with radioactivity, mixed it with cellular RNA and used this system to optimize the yield of the labeled microRNA. The solution was to dilute the total RNA before loading on a gel to avoid entrapment of microRNAs in large RNAs and residual RNA-protein complexes.

(b) Ligation of microRNA cDNAs into the topo-TA cloning vector was extremely inefficient. We initially suspected the adapters and ordered new adapters twice. Eventually the problem was traced to problems with the batch of topo-TA cloning vector that we had obtained from the commercial suppliers.

Task 1.3: De-convolute the sequences and do bioinformatics on public sequence databases to identify predicted and novel microRNAs.

Status: We have now sequenced 200 concatamers of microRNAs from LnCAP cells and have identified over 60 known microRNAs in the clones. Surprisingly, there are a high number of sequences in the concatamers that do not correspond to known microRNAs. We are currently evaluating whether these unknown sequences are from the human genome or are contaminants from bacteria or some other source. Because the sequences are short (less than 21 bases), single base mismatches with the genomic sequence (due to errors in base calling or due to

polymorphisms in the genomic sequence) make it possible that BLAST or other automated programs will merely report the absence of perfect matches to the genomic sequence and therefore call the sequences as "unknown". We are therefore manually examining whether the "unknown" sequences might match with known microRNAs after allowing for single-base mismatches.

New Task 1.5: This task was not proposed in the original project. Because of the difficulties of cloning microRNAs and because of the advances in labeling microRNAs such that they can be used on commercially arrayed microarrays, we have also explored whether microarrays can be used in parallel to identify large changes in specific microRNAs upon growth of LnCAP cells in the presence and absence of androgen. We are using a microRNA labeling and microarray kit from Invitrogen. The results are just beginning to come in from the first cycle of hybridization. We have identified over 20 microRNAs that are changed in level upon treatment with androgens, but the signals of most of the microRNAs are weak. We are therefore repeating the microarray hybridizations to see how reproducible the results are before embarking on task 2 with the differentially expressed microRNAs.

KEY RESEARCH ACCOMPLISHMENTS:

- Successfully troubleshooting the isolation and cloning of microRNAs
- Identification of single-base mismatches as a potential problem for the bioinformatics identification of cloned cDNAs as bona fide microRNAs.
- Adaptation of new commercially available microarrays of microRNAs to determine which microRNAs are changed upon treatment of LnCAP prostate cancer cells with androgens.

REPORTABLE OUTCOMES:

- None yet.

CONCLUSION:

We are still at an early stage of the project. Our renewed success with cloning microRNAs gives us confidence that we will succeed in identifying new microRNAs in prostate cancer cells. If we succeed in adapting the commercially available microarrays of microRNAs to measure changes in microRNA levels upon androgen treatment, we can make up the lost time and move quickly to task 2 where we focus on validating the microRNAs that are indeed changed by androgen treatment.

New microRNAs identified in prostate cancer cells will reveal new pathways of modulating gene expression in prostate cancer cells. Variation in the levels of microRNAs between different prostate cancer cells will give us an idea of the extent to which microRNA might explain the differences in phenotypes between prostate cancer cells. Identification of microRNAs that are up- or down-regulated by androgens in the androgen responsive LnCAP prostate cancer cell will reveal a new arm by which androgens regulate the repertoire of genes expressed in prostate cancer. Since androgens are a major etiological factor for prostate cancer

formation and since anti-androgen therapy is a bedrock for the treatment of prostate cancer, any new information on how androgens regulate gene expression will be of importance to prostate cancer.

REFERENCES: None

APPENDICES: None

SUPPORTING DATA: None